Serial Number: 09/521,524 Filing Date: March 8, 2000

Title: RAPID GENERATION OF RECOMBINANT ADENOVIRAL VECTORS

- 12. (Amended) The shuttle plasmid of claim 11, [wherein] <u>further consisting of PacI</u> restriction endonuclease sites [flank] <u>flanking</u> either end of the Ad sequences.
- 13. (Amended) The shuttle plasmid of claim 11, further [comprising] consisting of a multiple cloning site positioned between 1 and 9.2 map units.
- 14. (Amended) The shuttle plasmid of claim 11, [wherein the shuttle plasmid] further [comprises] consisting of a sequence encoding a gene of interest.
- 15. (Amended) The shuttle plasmid of claim 11, further [comprising] consisting of a promoter, or other sequence used to drive expression from a transgene.
- 16. (Amended) A cloning system for generating recombinant adenovirus comprising:
  - (a) an Ad backbone plasmid consisting [essentially] of an Ad genome lacking map units 0 to 9.2, wherein the numbering of the map units starts with the lefthand ITR and wherein the backbone plasmid lacks a loxP sequence, and
  - (b) a shuttle plasmid consisting [essentially] of Ad sequences from 0 to 1 map units and 9.2 to 16.1 map units of an Ad genome [wherein the shuttle plasmid lacks a loxP sequence].
- 17. (Amended) A host cell comprising:
  - (a) an Ad backbone plasmid [comprising] consisting of an Ad genome lacking map units 0 to 9.2, wherein the numbering of the map units starts with the lefthand ITR [and wherein the backbone plasmid lacks afloxP sequence], and
  - (b) a shuttle plasmid [comprising] consisting of Ad sequences from 0 to 1 map units and 9.2 to 16.1 map units of an Adgenome [wherein the shuttle plasmid lacks a loxP sequence].

22. (Amended) A method for producing recombinant adenovirus comprising contacting a host cell with

- (a) an Ad backbone plasmid [comprising] consisting of an Ad genome lacking map units 0 to 9.2, wherein the numbering of the map units starts with the lefthand ITR [and wherein the backbone plasmid lacks a loxP sequence], and
- (b) a shuttle plasmid [comprising] <u>consisting of</u> Ad sequences from 0 to 1 map units and 9.2 to 16.1 map units of an Ad genome [wherein the shuttle plasmid lacks a loxP sequence].
- 25. (Amended) The method of claim 22, wherein the shuttle plasmid further [comprises] consists of a sequence encoding a gene of interest.

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(NEW) The shuttle plasmid of claim 11, wherein any or all open reading frames constituting E4 have been modified in the backbone plasmid.

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(NEW) The shuttle plasmid of claim 26, wherein the modification is a substitution, insertion, or deletion of one or more nucleotides.

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(NEW) The shuttle plasmid of claim 11, wherein E3 has been modified in the backbone plasmid.

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(NEW) The shuttle plasmid of claim 28, wherein the modification is a substitution, insertion, or deletion of one or more nucleotides.

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(NEW) The shuttle plasmid of claim 28 wherein E3 has been modified to contain a multiple cloning site.

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(NEW) The shuttle plasmid of claim 28, wherein one or more genes required for Herpes Simplex Virus (HSV) packaging and an HSV origin of replication have been placed within the E3 region.

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(NEW) The shuttle plasmid of claim 11, further consisting in the backbone plasmid HSV Amplicon sequences required for packaging and replication.

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(NEW) The shuttle plasmid of claim 11, further consisting in the backbone plasmid one or more sequences that allow for integration of sequences into cells after viral infection.

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(NEW) The shuttle plasmid of claim 16, further consisting of PacI restriction endonuclease sites flanking either end of the Ad sequences.

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(NEW) The shuttle plasmid of claim 16, further consisting of a multiple cloning site positioned between 1 and 9.2 map units.

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(NEW) The shuttle plasmid of claim 16, further consisting of a sequence encoding a gene of interest.

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(NEW) The shuttle plasmid of claim 16, further consisting of a promoter, or other sequence used to drive expression from a transgene.

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(NEW) The host cell of claim 17, wherein the shuttle plasmid further consists of PacI restriction endonuclease sites flanking either end of the Ad sequences.

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(NEW) The host cell of claim 17, wherein the shuttle plasmid further consists of a multiple cloning site positioned between 1 and 9.2 map units.

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(NEW) The host cell of claim 17, wherein the shuttle plasmid further consists of a sequence encoding a gene of interest.

(NEW) The host cell of claim 17, wherein the shuttle plasmid further consists of a promoter, or other sequence used to drive expression from a transgene.

(NEW) The host cell of claim 17, wherein any or all open reading frames constituting E4 have been modified in the backbone plasmid.

(NEW) The host cell of claim 42, wherein the modification is a substitution, insertion, or deletion of one or more nucleotides.

(NEW) The host cell of claim 17, wherein E3 has been modified in the backbone plasmid.

(NEW) The host cell of claim 44, wherein the modification is a substitution, insertion, or deletion of one or more nucleotides.

(NEW) The host cell of claim 44, wherein E3 has been modified to contain a multiple cloning site.

(NEW) The host cell of claim 44, wherein one or more genes required for Herpes Simplex Virus (HSV) packaging and an HSV origin of replication have been placed within the E3 region.

(NEW) The host cell of claim 17, further consisting in the backbone plasmid HSV Amplicon sequences required for packaging and replication.

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(NEW) The host cell of claim 17, further consisting in the backbone plasmid one or more sequences that allow for integration of sequences into cells after giral infection.

(NEW) The method of claim 22, wherein the shuttle plasmid further consists of PacI restriction endonuclease sites flanking either end of the Ad sequences.

(NEW) The method of claim 22, wherein the shuttle plasmid further consists of a multiple cloning site positioned between 1 and 9.2 map units.

(NEW) The method of claim 22, wherein the shuttle plasmid further consists of a promoter, or other sequence used to drive expression from a transgene.

(NEW) The method of claim 22, wherein any or all open reading frames constituting E4 have been modified in the backbone plasmid.

(NEW) The method of claim 53, wherein the modification is a substitution, insertion, or deletion of one or more nucleotides.

(NEW) The method of claim 22, wherein  $\mathbb{R}^3$  has been modified in the backbone plasmid.

(NEW) The method of claim 55, wherein the modification is a substitution, insertion, or deletion of one or more nucleotides.

(NEW) The method of claim 55, wherein E3 has been modified to contain a multiple cloning site.

(NEW) The method of claim 55, wherein one or more genes required for Herpes Simplex Virus (HSV) packaging and an HSV origin of replication have been placed within the E3 region.